

The Sampling and Analysis of Human Livers

Rolf Zeisler

National Bureau of Standards, Gaithersburg, MD 20899

Accepted: January 9, 1986

A comprehensive approach to the analysis of human livers has been developed in a pilot program for a National Environmental Specimen Bank (NESB). Since 1980, the pilot NESB program has examined the collection, processing, storage and analysis of human livers. Sampling protocols, handling procedures and analytical methods have been developed and implemented considering the requirements for valid analytical results. Sampling and handling included the use of cleanroom technology, specific clean implements and packing materials made from titanium and Teflon and flash-freezing and preservation at liquid nitrogen temperature. Neutron activation analysis played a major role in the implemented analytical scheme. The scheme combined up to four analytical techniques to determine the distribution of 29 trace elements in 66 human livers.

Key words: analysis; human liver; low temperature preservation; neutron activation analysis; sampling; specimen banking; trace elements.

1. Introduction

1.1 Biomonitoring for Environmental Health

Monitoring is used to determine the present state of the environment with respect to concentrations of a variety of hazardous materials. Focusing on the biosphere, biomonitoring is the observation of symptoms, including both the registration of biological effects and the determination of trends in residual or accumulated hazardous substances. In this context, the monitoring of trends in pollutant loadings may provide the most comprehensive insight into interrelations between pollutant burden and environmental health.

About the Author: Rolf Zeisler is with the Center for Analytical Chemistry in NBS' National Measurement Laboratory. The work he describes was supported in part by the Office of Research and Development, U.S. Environmental Protection Agency.

Biomonitoring of pollutant loading is an extremely complex task and requires a systematic approach to all aspects of a monitoring program. This includes the scientific evaluation of suitable biological specimens, their appropriate collection and sampling for analysis, the analytical methodology capable of obtaining the desired information, the dissemination of data, and the long-term storage of specimens for retrospective analysis. The requirements, experiences, and developments in monitoring environmental materials and specimen banking have been discussed in three recent international workshops [1–3]¹ and have resulted in recommendations for the implementation of specimen banking programs related to biomonitoring. In concurrence with these efforts, two pilot programs for the implementation of an environmental specimen bank have been initiated [4,5]. The pilot programs provide the basic information needed for a more formalistic approach to the complex task of biomonitoring and specimen banking.

¹ Figures in brackets indicate literature references.

1.2 U.S. Pilot National Environmental Specimen Bank Program

The historical development in the United States during the 1970's of the concept of a National Environmental Specimen Bank (NESB) has been reviewed by Goldstein [6,7]. The purpose of such a national system is 1) to detect changes in the environment on a real-time basis (i.e., monitoring) using bioaccumulators as indicators, 2) to distinguish such changes from natural inputs, and 3) to provide a "bank" of well preserved and documented environmental samples for retrospective analyses in future years as analytical techniques improve or as new pollutants are identified.

Since 1975, the National Bureau of Standards (NBS), in conjunction with the U.S. Environmental Protection Agency (EPA), has been involved in research relating to the establishment of a National Environmental Specimen Bank. The initial plans and results of the EPA/NBS effort for the NESB in research and methodology evaluation have been described [8-13].

In 1979, a special "clean" laboratory/storage facility [14] was completed at NBS to initiate a *Pilot* National Environmental Specimen Bank Program. This pilot program is designed to evaluate the feasibility of a national program by providing actual working experience in all aspects of specimen banking, i.e., specimen collection, processing, storage, analysis, and data management. The major goals of this pilot study are: 1) to develop analytical protocols for sampling, processing, and storing four types of environmental accumulators and biomonitors; 2) to evaluate and improve analytical methods for the determination of both trace element and organic pollutants in biological matrices; 3) to evaluate the feasibility of long-term storage under various conditions; and 4) to provide a "bank" of samples for retrospective analyses in future years. The experience gained during the pilot study will be the basis for evaluating the feasibility of establishing a National (or International) Environmental Specimen Bank.

Four types of accumulators and biomonitors were selected for inclusion in the NBS pilot program as a result of an EPA/NBS Workshop on "Recommendations and Conclusions on the National Environmental Specimen Bank" held in 1976 [8]. These accumulators are: 1) human soft tissue—liver, 2) aquatic accumulator—marine bivalves and sediments, 3) food monitor—total diet composite, 4) air pollutant accumulator. Human liver was selected as the first sample type for the inclusion in the pilot specimen banking program. Sample types 2 and 3 are currently incorporated into the program with continued collection, storage, and analysis of the previous sample type. Consequently, the

major efforts in the NESB have been concentrated on the development of the human liver as a monitor tissue. This contribution summarizes this experience with specific emphasis on sampling and analysis by nuclear methods.

2. Development of the Human Monitor Tissue

2.1 Criteria Determining the Selection of Monitor Tissues

For the selection and development of a monitor tissue the following aspects have to be considered: 1) tissues are selected because they are known either to respond to pollutant exposure or to accumulate pollutants; 2) a choice has to be made between biopsy and autopsy samples; 3) the quantity of material must be sufficient for baseline determination of constituents and storage for future analysis; 4) it must be possible to collect biologically or anatomically well described samples; and 5) analytical methods must be available to determine the pollutants. Table 1 lists a selection of possible monitor tissues and their evaluation according to the above considerations.

The general advantage of monitor tissue taken by biopsy versus autopsy is the possibility to monitor pollutant trends in the very same individual. The individual would reflect his own habitat over an extended time period. However, a wide variety of tissues is not available for sequential collection throughout the lifetime of an individual. Most of the biopsy tissue samples are collected in connection with surgical incisions, which usually provide only small samples, or one time events (e.g., placenta) where ethical barriers restrict the degree of sampling. Some monitor tissues can be collected on a routine basis without the above restrictions. Non-intrusive sampling includes hair, nail clippings, and various excretions. Requiring only minimal intrusion, blood has been demonstrated to be useful as an effective monitor that responds directly to the environment. In the U.S., the large-scale screening of blood from children helped to verify the accumulation of lead in blood and consequently resulted in the initiatives to lower the risk of exposure, e.g., by banning the use of lead based paints and reducing the lead content of gasoline [15,16]. The disadvantage of blood as a monitor is the fact that it reflects more acute exposure rather than being an accumulator. The natural dilution effect resulting from the blood's function as a distributor means that the concentrations of most pollutants are very low and difficult to measure. The danger of contamination during collection is very high (because of the low concentrations), although this problem can be minimized [17]. A more serious problem is the preservation of the blood sample and its long term storage without change, e.g., coagulation.

Table 1. Criteria for the selection of human monitor tissue.

Key: (1) highly favorable/no or little difficulties; (2) less favorable/limitations and problems; (?) questionable; and (0) no information.

	Sampling	Pollutant Accumulator	Sample Size for Banking	Biological Characterization	Sampling Characteristics	Inorganic Analysis	Organic Analysis
	Blood	?	1	2	1	2	1
	Milk	2	1	2	1	1	1
	Hair	1	2	1	?	2	0
	Nail	1	2	1	2	1	0
In Vivo							
	Teeth	1	2	1	?	2	0
	Placenta	2	1	1	2	1	2
	Feces	?	1	2	2	1	2
	Urine	?	1	2	1	1	1
	Brain	1	1	1	2	1	2
	Liver	1	1	1	2	1	2
	Lung	1	1	1	2	1	2
	Kidney	1	1	1	2	1	2
Post Mortem							
	Adipose	1	1	1	2	2	1
	Bone	1	1	1	2	1	0
	Muscle	1	1	1	2	1	2

2.2 Human Liver as Monitor

2.2.1 Biological and Anatomical Aspects. The liver exhibits several features which are advantageous to its use as a human monitor tissue. The liver is among the few organs which are significantly exposed to most of the pollutants that enter the body. Practically all substances which are assimilated by the human body are transported through the blood stream and consequently pass through the liver. The liver's function is to detoxify, store, and regulate trace substances in the body. Comparative studies on biological half-times [18] of trace substances in tissues support the storage function of the liver. Though some toxic elements have shown more affinity to calcareous tissue, biological half-times of trace substances in liver are so long that liver can be regarded as a general accumulator. In addition, the liver accumulates lipophilic organic trace substances because of its fat content. Therefore the liver is possibly the most universal accumulator tissue.

For the purpose of sample collection and specimen banking, the liver offers a biologically and anatomically well defined tissue, which also can be obtained in large quantities. The collection of the left lobes of the liver alone provides 200–400 g per sample depending on the physical stature of the subject. The liver is homogeneous in its function and in its macroscopic structure. During an autopsy, only a few cuts of ligaments and blood vessels are necessary to remove the liver from the corpse. The integrity of the sample can thus be pre-

served and subsequent sampling can be carried out under controlled conditions.

2.2.2 Sampling Protocol Development. Because of the extremely low concentrations of trace element and organic pollutants found in most environmental samples, extreme caution must be exercised during sample collection and sample processing to avoid contamination. A detailed sampling protocol, designed to provide samples suitable for both trace element and organic analysis, has been developed and implemented for the collection of human liver samples. For the complete detailed protocol see Harrison et al., [5,19]. This liver sampling protocol was developed in conjunction with those individuals performing the autopsies, and its implementation within the bounds of practicality required periods of education and close cooperation. The need for careful communication was evident from perceived meanings of such terms as "clean," i.e., interpreted as "sterile" by autopsy personnel vs. "non-contaminated chemically" by analytical chemists.

The sampling protocol was designed to avoid possible contamination of the sample by either inorganic or organic constituents. Teflon supplies (e.g., sheets, bags, and storage jars) were selected as the most suitable materials to prevent contamination of the sample from inorganic and organic constituents and from the diffusion of water [7,15]. The protocol specifies the use of such non-contaminating items as non-talced vinyl gloves, pre-cleaned dust-free Teflon FEP sheets and bags, high-purity water, and a titanium/Teflon TFE knife. These

items are provided by NBS at each collection site to insure uniformity in sampling materials. A special knife with a titanium blade and a Teflon TFE handle was designed and constructed at NBS for use during dissecting of the specimen. This special knife is used to avoid trace element contamination by various constituents associated with a regular stainless-steel scalpel/knife (e.g., Ni and Cr) and to limit the possible contamination by an element of little environmental interest, namely titanium. The liver samples are sealed in Teflon FEP bags, frozen in liquid nitrogen (LN₂), and shipped to NBS in a special biological shipper at LN₂ vapor temperature.

To eliminate potentially infectious liver samples from the specimen bank, a blood sample is removed from the donor at the time of the autopsy to be used for hepatitis B screening. In addition, liver specimens from the right lobe are removed for preparation of histological slides. These slides are examined by a pathologist to verify the absence of infectious diseases and then stored at NBS. They are also intended to serve for possible future reference.

A data form, sent to NBS with each liver sample, contains information about the donor, e.g., date of birth, sex, residence, ethnic group, height, weight, smoking history, occupation (if known), date and time of death and autopsy, diagnosis of autopsy, and liver specimen weight (see ref. [18,19]).

During the first 18 months, 300 liver samples from three geographical locations (Baltimore, MD; Minneapolis, MN; and Seattle, WA) were collected using this protocol. During the remaining years of the pilot study, liver samples were received predominately from one location (Seattle) at a rate of approximately 50 per year, bringing the number of banked specimens to about 500. After the first year of human liver collection, the sample collection protocol was evaluated with respect to such items as: initial set-up costs for each site, sample procurement costs, transport time, time required for receipt of hepatitis results and histological slides, and suitability of the donor selection criteria [20].

A conclusion from the experience of the first year of collection was that a technician was needed at the collection site whose primary responsibility was to work closely with autopsy personnel to select, remove, and prepare the liver samples. Since these technicians were responsible for implementing the sampling protocol, NBS personnel worked closely with them to stress the importance of following the protocol precisely. NBS must have confidence that the samples stored in the specimen bank are collected exactly as prescribed in the protocol.

2.2.3 Analytical Aspects. The U.S. Pilot NESB Program, and possibly any biomonitoring program, has

to operate under constraints regarding the amount of available material from which the desired set of data will be obtained. In addition, modern analytical techniques require only small subsamples for analysis. Since significant trace element inhomogeneities have been found within human livers [21,22], homogenization of the bulk sample is required before analysis. The necessary reduction of a bulk sample to a laboratory sample suitable for analytical techniques can introduce errors due to contamination and heterogeneity. These errors may become the limiting factors for achieving the goal of precise and accurate analysis [23]. As the analytical test portion becomes smaller, better homogeneity is required so that the sample is representative of the bulk. Consequently, a major research effort of the pilot program has focused on homogenization procedures to provide uncontaminated analytical increments which represent the sample and which are homogeneous for subsampling of the analytical test portions.

Though the technique of cryogenic homogenization (brittle fracture technique) was first introduced and evaluated by Iyengar [24], considerable upgrading and evaluation was required before it could be routinely applied in the pilot program. The initial sample capacity of about 20 g was too small for application to the NESB program. Sample sizes for the human liver specimens in the pilot program are 120–180 g; and sample sizes up to 1000 g are anticipated for future specimens. As part of the pilot program, NBS has developed and evaluated the required technology for homogenizing larger samples [25].

NBS has designed two larger Teflon ball mills with capacities of 60 g and 150 g. A performance evaluation of the ball mills, based on particle size distribution and mixing ability, led to the design of the disk mill which has been determined to give superior performance and which is adequate for our needs (sec. 3.1). These experimental designs and their features are listed in table 2. Extensive evaluation for liver tissue showed that the cryogenic homogenization procedure with Teflon disk mills can be readily applied to soft tissues. Applications to other tissues resulted in equally fine particulate homogenates, using sieves for quick assay of the results of the homogenization process (fig. 1). These results were confirmed with the determination of sampling constants (sec. 3.1).

An important criterion for a comprehensive biomonitoring program is the availability of analytical techniques which can detect and determine all substances of concern at reasonable cost. The human liver, owing to its function as an accumulator, has higher concentrations of many elements and compounds of interest than most other tissues and therefore has less need for ultratrace level techniques. For most of the elements of

Table 2. Cryogenic homogenization machines used in the Pilot NESB Program.

	Teflon Milling Chamber Volume		Capacity for Soft Tissue	Drive Unit	
	Total	Milling Body		Type	Motor Power/Revolutions
Ball Mill A	920 cm ³	ball, 65 cm ³	60 g	NBS shaker	0.25 kW, variable
Ball Mill B	1000 cm ³	ball, 200 cm ³	150 g		
Disk Mill #1	1400 cm ³	disk and 1 ring, 700 cm ³	100 g	Shatter box by Spex Industries	0.25 kW, 850 rpm
Disk Mill #2	2500 cm ³	disk and 2 rings, 1300 cm ³	250 g	Disk Mill TS 250 by Siebetchnik	0.75 kW, 1000 rpm
Disk Mill #3	6500 cm ³	disk and 3 rings, 3100 cm ³	~1000 g		

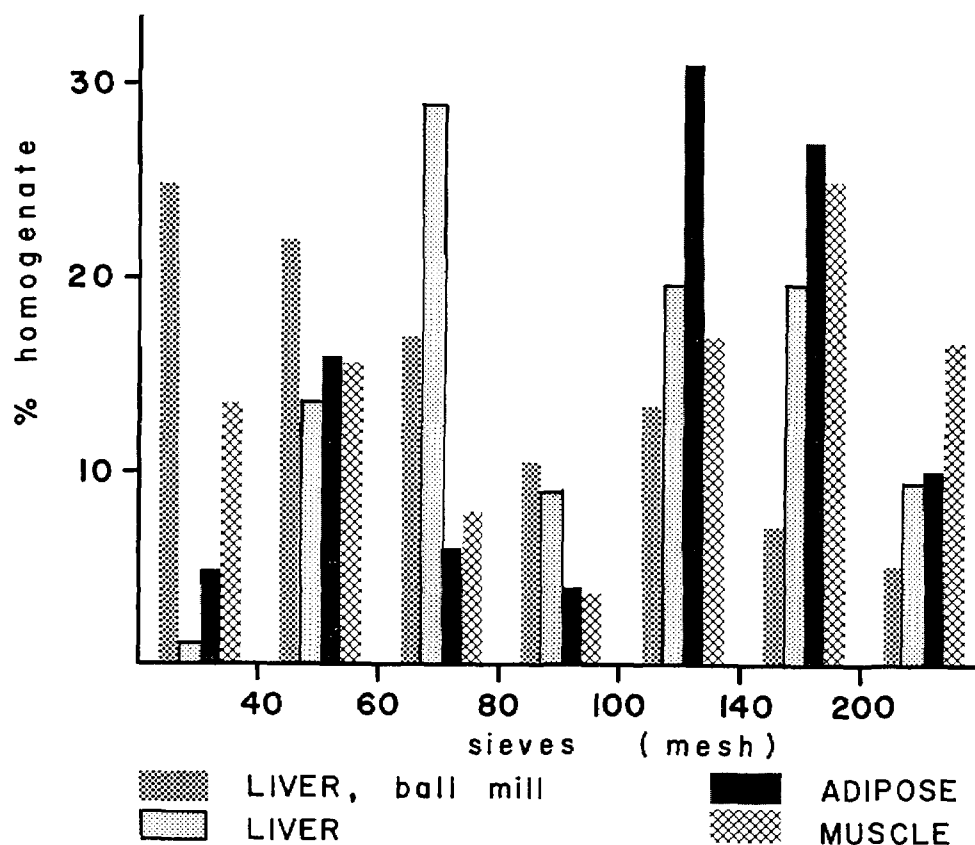


Figure 1-Particle size distribution of frozen tissue after homogenization in Teflon mills: approximately 150 g of tissue, grinding time 4 min. liver in ball mill B, and liver, adipose, and muscle tissue in disk mill no. 2.

interest [2,26], several routine techniques are available. Nuclear techniques can provide a broad data base on the liver (sec. 4) since no matrix interference is encountered. Furthermore, the liver tissue is easily ashed for techniques, including radiochemistry, that require wet chemistry before analysis.

In the U.S. Pilot NESB Program these basic conditions led to the implementation of a comprehensive analytical protocol, using four techniques for the deter-

mination of 31 trace elements of biological and toxicological importance [26]. This protocol is illustrated in figure 2. The quality of the analytical data is ensured by several measures. All analytical techniques used have demonstrated their capabilities during previous certification analyses of biological Standard Reference Materials (SRMs). Frequent parallel analyses of SRMs throughout the analytical program ensured high quality. Furthermore, it was possible to determine

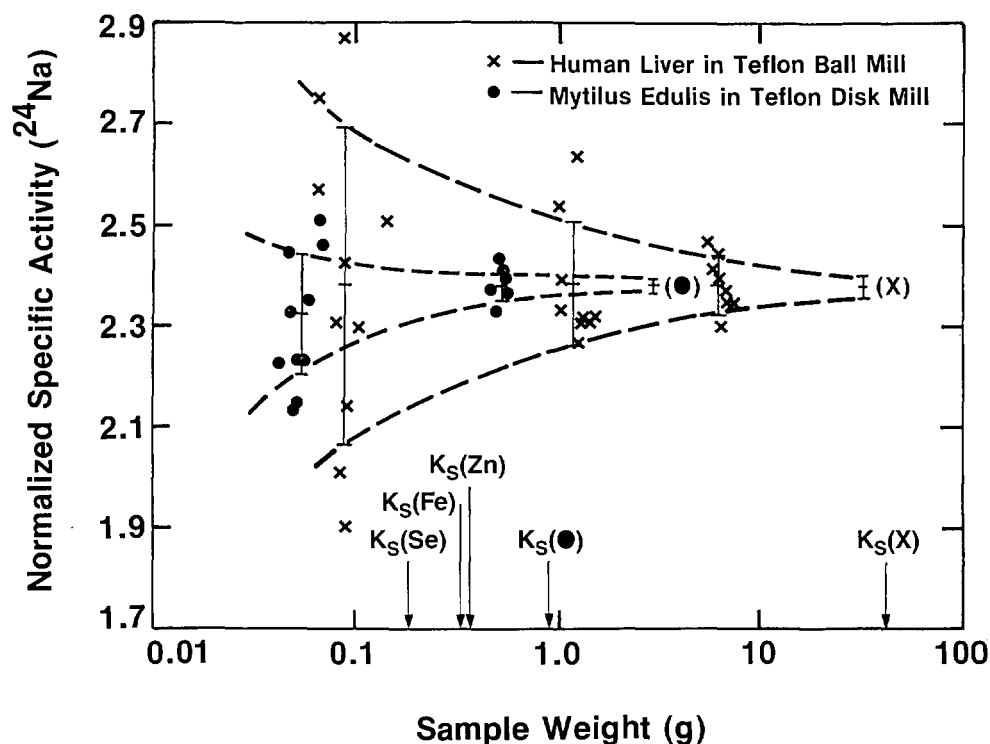


Figure 3—Sampling diagram of ^{24}Na in human liver and mussel homogenate. Specific activity (cts $\text{g}^{-1} \text{s}^{-1} \cdot 10^{-2}$) scale is for the liver homogenate; values for mussel homogenate are normalized to the liver values for comparison.

be the cause of significant errors due to sample inhomogeneity. The inadequate performance of the ball mill resulted in the design of a disk mill which yielded a K_s of 0.95 g. Thus, using the disk mill, the analytical data obtained for a 1 g sample will depend on the precision of the analytical techniques rather than on sampling uncertainty.

This homogeneity evaluation was supplemented by the actual analysis of a considerable number of subsamples from a homogenized human liver specimen. Using PGAA and INAA for major and trace element composition, 10 randomly prepared subsamples (nominally 1 g) of the homogenate were analyzed. In all concentration ranges, from $10^5 \mu\text{g/g}$ for nitrogen to $10^{-4} \mu\text{g/g}$ for scandium, no sample inhomogeneity was observed; the sampling error being dominated by counting statistics. The elements Na, Br, Zn, and Fe, with small errors due to counting statistics, showed coefficients of variation $\leq 1\%$ for 1 g samples. This is in good agreement with the tracer experiment, though better data than those obtained for the bulk sample with its introduced gross inhomogeneity could be expected if the analytical error could be reduced.

The application of nuclear techniques to this problem has helped considerably to improve the cryogenic homogenization procedures. Though limited in their precision due to the radioactive decay, tracer techniques and instrumental multi-element determination provided useful information about the applied system.

3.2 Comprehensive Activation Analysis Scheme for Human Liver Analysis

An activation analysis scheme has been developed which is efficient in the use of sample material as well as in total cost and which provides a maximum amount of data on a routine basis. The procedure can be used to determine 33 elements in a specific subsample of the human liver. It consists of PGAA for the determination of H, B, C, N, Na, P, S, Cl, K and Cd and INAA for the determination of the elements Na, Mg, Al, Cl, K, V, Cr, Mn, Fe, Co, Cu, Zn, As, Se, Cr, Rb, Mo, Ag, Cd, Sb, Cs, La, Ce, Sm, Au, and Hg, followed by a RNAA procedure for Sn.

The liver homogenates are freeze-dried before use for activation analysis. Approximately 250 g of dry material (corresponding to about 1 g fresh weight) is pressed into pellets and irradiated. An overnight count is used for the PGAA determination. Short (1 min) and long (4 h) irradiations, with two counts each after suitable decay intervals, are needed for the INAA. After obtaining all instrumental data, the sample is dissolved and ^{119}Sn is radiochemically separated using liquid/liquid extraction [31]. Activation analysis is very suitable for the types of analyses encountered in this biomonitoring program. Due to the variations in elemental concentrations encountered in human liver samples, a technique must cover a broad dynamic range without significant changes in precision and accuracy. Activa-

tion analysis is largely independent of changes in concentration unless the detection limit of the technique is approached. The analysis of the 66 human liver specimens that were collected during 1980 through 1982 showed that for several elements the detection limit of the applied instrumental procedure was approached when concentrations lower than previously reported [32] were found. The effects of this are demonstrated in figure 4, which shows that the precision of the cadmium analysis dependent on the concentration of the element. With the current activation analysis procedure, cadmium concentrations cannot be determined reliably below 1 $\mu\text{g/g}$. Similarly, the detection limit was reached in the majority of samples for the elements V, Cr, and As. A Compton Suppressor Spectrometer, recently installed at NBS, will be used for future analyses of the liver specimens. This new instrumentation will provide a considerable increase in sensitivity for the determination of these elements. In addition, a previously developed radiochemical separation procedure can be implemented to obtain accurate high precision data on Cr, Cu, As, Se, Mo, Ag, Cd, and Sb [3].

4. Value of Specimen Banking Program for Biomonitoring Environmental Health

4.1 Baseline Data

The process of obtaining reliable, high quality data requires the careful planning, investigation, and evaluation of every step in a chemical measurement system. To achieve this goal, the collaboration of scientists from the different disciplines involved is needed. This cooperation must start from the very beginning of a bio-

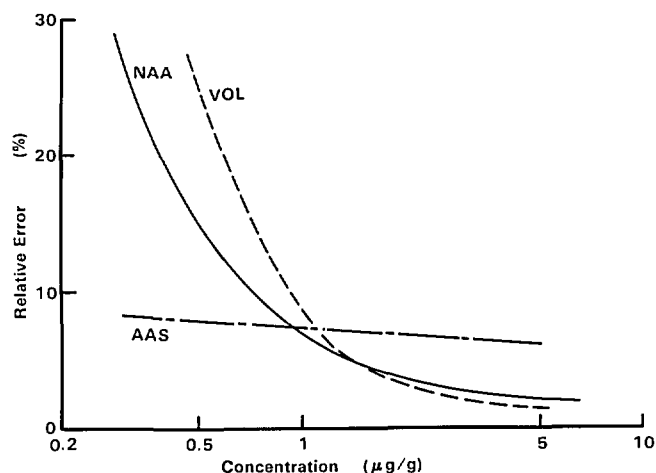


Figure 4—Imprecision of the analytical techniques under the constraints of the analytical protocol as observed for the determination of cadmium in human liver.

monitoring and specimen banking program. Just as the environmental ecologist must be aware of the measurement strategy and its benefits and limitations, the relationship between the analytical signal and the original question must be maintained. A formal specimen banking program for biomonitoring provides the necessary framework for such cooperation and consequently will achieve a credible data bank as a foundation for valid scientific conclusions. The current pilot programs have implemented this strategy and first results support this general approach.

The individual concentrations of 31 trace elements in 66 human liver specimens are summarized in figure 5. This figure is a plot of the more than 2,000 data points obtained in the first year's analysis of the human livers and also includes the concentration ranges of the elements reported in the literature and compiled by Iyengar for human livers [32].

The outstanding result of this study is the very narrow range of data obtained for many essential trace elements. There is only a factor of 1.8 difference between the lowest and highest value for selenium; other essential trace elements (Mg, Mn, Co, Cu, Zn) vary by a factor of 2 to 3.5. In sharp contrast to these findings for essential elements is the scatter of pollutant element concentrations (Al, As, Cd, Hg, Pb, Tl) over orders of magnitude. Since the biological variability of the individual specimens appears to be reasonably small, these broad ranges for the pollutant elements in the NESB data may point to sources of pollution to which the individuals were exposed.

When compared to the literature data [32], the elemental concentrations found in the liver samples, especially for pollutant trace elements, are at the lower side or even below the previously reported data. If these trends cannot be explained by the selection of the specimens and, for example, their low exposure to pollutant sources, then these results can be interpreted as a consequence of the implemented chemical measurement system. The precautions taken to preserve the integrity of the samples, especially the exclusion of possible sources of contamination during sampling and sample preparation, would naturally result in lower concentrations.

Further conclusions, especially on environmental impacts, are not readily available because the data base at this time is much too small. However, some interesting findings can be pointed out to illustrate the potential utility of a specimen banking program. The spread of the selenium data is much lower if individuals from the same geographical area are studied. When compared to the data obtained on samples from Seattle and Minneapolis, donors selenium levels in individuals from Baltimore are low. The average of the data from Baltimore is 4.4 $\mu\text{g/g}$,

Human Liver Data

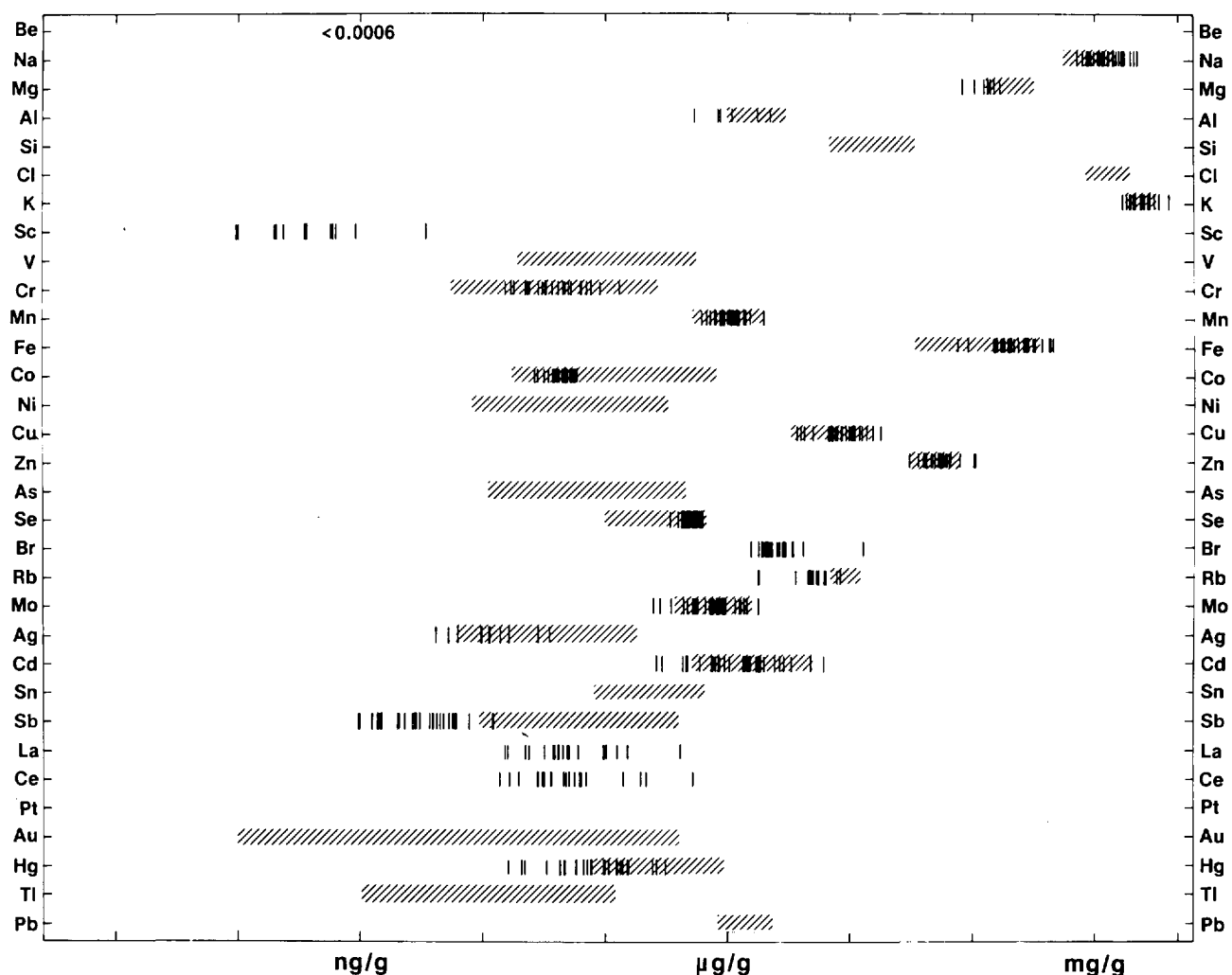


Figure 5—Elemental concentrations in 66 individual liver specimens and the concentration ranges reported in the literature [32]. Data from the pilot specimen bank program denoted by one line for each data point. Literature ranges denoted by the shaded areas.

the Seattle average is $5.6 \mu\text{g/g}$, and the Minneapolis average is $6.1 \mu\text{g/g}$. Because of concern about possible analytical error, we have also investigated some data points which exceeded the previously reported ranges. The highest iron value of $522 \mu\text{g/g}$ was from a 40-year steel worker, while the highest zinc value of $96.1 \mu\text{g/g}$ was found in the liver of a galvanizing plant worker.

4.2 Organized Assessment of Environmental Health

From the experience in the ongoing pilot programs, several advantages to formalized specimen banking and biomonitoring can be pointed out. A major aspect is that experience and expertise are easily transferable between programs which follow the same recommended criteria for their development. Duplication of effort has been

avoided and by adopting common procedures, efficiency in implementation has been achieved. This principle is adaptable to a program having a few participants as to a program of global proportions.

Internationalization and/or inter-institutionalization of the banking and biomonitoring efforts is likely to provide the highest level of quality control and to make results comparable between institutes as well as nations. This is obviously essential for monitoring and assessing the true global burden from pollutants. Currently, samples are exchanged for quality assurance and analysis between the two pilot programs. In a completely transmissible system, specimens can be analyzed by the laboratory which is best qualified for the specific task.

Although the feasibility of long-term specimen storage for retrospective analysis has not been established, preliminary analysis after one year of storage does not

indicate any changes in trace element concentrations for livers stored under the specific conditions of the U.S. Pilot NESB. It is without doubt necessary that bio-monitoring programs need to comprise specimen storage for retrospective analysis. If specimens from previous lead analyses in Baltimore [36] had been collected and stored under the NESB protocol, it would be easy to determine if the decrease in concentrations by an order of magnitude is a realistic trend or a result of improved analytical methodology. If long-term stability can be guaranteed, banked specimens are far superior than data alone.

References

- [1] Berlin, A.; A. H. Wolff and Y. Hasegawa, The use of biological specimens for the assessment of human exposure to environmental pollutants. The Hague, The Netherlands. Martinus Nijhoff Publishers, 368 pp. (1979).
- [2] Luepke, N.-P., Monitoring environmental materials and specimen banking. The Hague, The Netherlands, Martinus Nijhoff Publishers, 591 pp. (1979).
- [3] Lewis, R. A.; N. Stein and C. W. Lewis, Environmental specimen banking and monitoring as related to banking, Boston, MA, U.S.A., Martinus Nijhoff Publishers, 358 pp. (1984).
- [4] Boehringer, U., Umweltprobenbank. Berlin, FRG, Umweltbundesamt. 2 Vol. 485 pp. (1981).
- [5] Harrison, S. H.; R. Zeisler and S. A. Wise, Pilot Program for the National Environmental Specimen Bank—Phase I. EPA-600/1-81-025. U.S. Environmental Protection Agency, 54 pp. (1981).
- [6] Goldstein, G. M., Plan for a National Environmental Specimen Bank, EPA-600/1-78-022. U.S. Environmental Protection Agency, 27 pp. (1978).
- [7] Goldstein, G. M. The National Environmental Specimen Bank—Its concepts, its history and its objectives. In: The Use of Biological Specimens for the Assessment of Human Exposure to Environmental Pollutants, A. Berlin, A. H. Wolff, Y. Hasegawa, eds., The Hague, The Netherlands, Martinus Nijhoff Publishers, p. 165 (1979).
- [8] Rook, H. L., and G. M. Goldstein, The National Environmental Specimen Bank. NBS Special Publ. 501, Washington, DC, U.S. Government Printing Office, 54 pp. (1978).
- [9] Rook, H. L., and P. D. Lafleur, Establishing and operating a specimen bank for environmental monitoring. In: The Use of Biological Specimens for the Assessment of Human Exposure to Environmental Pollutants, A. Berlin, A. H. Wolff, Y. Hasegawa, eds., The Hague, The Netherlands, Martinus Nijhoff Publishers, p. 309.
- [10] Gills, T. E., and H. L. Rook, Specimen bank research at the National Bureau of Standards to insure proper scientific protocols for sampling, storage, and analysis of environmental materials. In: Monitoring Environmental Materials and Specimen Banking, N.-P. Luepke, ed., The Hague, The Netherlands, Martinus Nijhoff Publishers, p. 263 (1979).
- [11] Maienthal, E. J., and D. A. Becker, A survey of current literature on sampling, sample handling, and long-term storage for environmental materials, NBS Tech. Note 929. Washington, DC, U.S. Government Printing Office, 34 pp. (1976).
- [12] Becker, D. A., and G. J. Maienthal, Evaluation of the National Environmental Specimen Bank Survey, EPA-600/1-77-015, U.S. Environmental Protection Agency, 269 pp. (1977).
- [13] Gills, T. E., H. L. Rook, The National Environmental Specimen Bank Research Program for Sampling, Storage, and Analyses, EPA-600/1-79-017, U.S. Environmental Protection Agency, 59 pp. (1979).
- [14] Moody, J. R., Design principles and construction of the pilot specimen bank facility. In: Pilot Program for the National Environmental Specimen Bank—Phase I. S. H. Harrison, R. Zeisler, S. A. Wise, eds., EPA-600/1-81-025, U.S. Environmental Protection Agency, p. 8 (1981).
- [15] Billik, H. I., A. S. Curran and D. R. Shier, Analysis of pediatric blood lead levels in New York City for 1970-1976, Environ. Health Persp. **31**, 183 (1979).
- [16] Billik, I. H.; A. S. Curran and D. R. Shier, Relation of pediatric blood levels to lead in gasoline, Environ. Health Perspect. **34**, 213 (1980).
- [17] Versieck, J. M. J., and A. B. H. Specke, Contamination induced by collection of liver biopsies and blood. In: Nuclear Activation Techniques in the Life Sciences, Vienna, IAEA-SM157, p. 39 (1972).
- [18] Sugita, M., The biological half-time of heavy metals, Int. Arch. Occup. Environ. Health **41**, 25 (1978).
- [19] Harrison, S. H., T. E. Gills, E. J. Maienthal, H. L. Rook, S. A. Wise, and R. Zeisler, The National Environmental Specimen Bank Pilot Program. In: Trace Substances in Environmental Health—XIV, D. D. Hemphill, ed., Columbia, MO, University of Missouri, p. 329 (1980).
- [20] Wise, S. A., K. A. Fitzpatrick, S. H. Harrison, and R. Zeisler, Operation of the U.S. Pilot National Environmental Specimen Bank Program. In: Environmental Specimen Banking and Monitoring as Related to Banking, R. A. Lewis, et al., eds., Boston, MA, U.S.A., Martinus Nijhoff Publishers, p. 108 (1984).
- [21] Lievens, P.; J. Versieck, R. Cornelis, and J. Hoste, The distribution of trace elements on normal human liver determined by semi-automated radiochemical neutron activation analysis, J. Radioanal. Chem. **37**, 483 (1977).
- [22] Schicha, H.; V. Kasperek, L. E. Riedel, K. Feinendegen, W. Vyska, and W. Müller, Trace elements in normal mammalian tissue and corresponding malignant tumors. In: Nuclear Activation Techniques in the Life Sciences, International Atomic Energy Agency, Vienna, 451 (1972).
- [23] Kratochvil, B., and J. Taylor, Sampling for chemical analysis, Anal. Chem. **53**, 924A.
- [24] Iyengar, G. V., and K. Kasperek, Application of the brittle fracture technique to homogenize biological samples and some observations regarding the distribution behavior of the trace elements in different concentration levels in a biological matrix, J. Radioanal. Chem. **38**, 301 (1977).
- [25] Langland, J. K.; S. H. Harrison and R. Zeisler, Cryogenic homogenization of biological tissues, Anal. Chem. **55**, 2431 (1983).
- [26] Zeisler, R.; S. H. Harrison and S. A. Wise, Analysis of human liver specimens in the U.S. Pilot National Environmental Specimen Bank Program. In: Environmental Specimen Banking and Monitoring as Related to Banking, R. A. Lewis et al. eds., Boston, MA, U.S.A., Martinus Nijhoff Publishers, p. 331 (1984).
- [27] Moody, J. R., and R. M. Lindstrom, Selection and cleaning of plastic containers for storage of trace element samples, Anal. Chem. **49**, 2264 (1977).
- [28] Lutz, G. E., J. S. Stemple and H. L. Rook, Evaluation by activation analysis of elemental retention in biological samples after low temperature ashing, J. Radioanal. Chem. **39**, 277 (1977).

- [29] Visman, J., A general sampling theory, *Mat. Res. Stds.* **9**, 8 (1969).
- [30] Ingamells, C. O., and P. Switzer, A proposed sampling constant for use in geochemical analysis, *Talanta* **20**, 547 (1973).
- [31] Greenberg, R. R., Improved radiochemical procedure for tin determination. In: *Pilot Program for the National Environmental specimen Bank—Analysis of Human Liver Specimens*, NBS Spec. Publ. 656, Washington, DC, U.S. Government Printing Office, p. 9 (1983).
- [32] Iyengar, G. V.; N. E. Kollmer and H. J. M. Bowen, *The elemental composition of human tissue and body fluids*, Verlag Chemie, Weinheim: New York.
- [33] Gallorini, M.; R. R. Greenberg and T. E. Gills, Simultaneous determination of arsenic, antimony, cadmium, chromium, copper, and selenium in environmental material by radiochemical neutron activation analysis, *Anal. Chem.* **50**, 1979 (1978).
- [34] Poklis, A., and H. C. Freimuth, Lead distribution in soft tissues of Baltimore residents, *Bull. Environ. Contam. Tox.* **15**, 311 (1976).